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CIRCADIAN RHYTHMS IN HUMAN SALIVARY FLOW RATE AND COMPOSITION

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SUMMARY

1. Unstimulated whole saliva and parotid saliva stimulated at a constant flow rate of 1·0 ml./min were collected from eight subjects at about 07.00, 11.00, 14.00, 17.00 and 22.00 hr and oral temperature was recorded several times daily for time spans of between 4 and 26 days. A least-squares cosine wave was fitted to the data to test for the presence and characteristics of circadian rhythms.

2. Estimates of mean level, amplitude, acrophase and period were obtained for different components and the results were subjected to cosinor analysis.

3. Unstimulated whole saliva showed significant circadian rhythms in flow rate and in the concentrations of sodium and chloride but not in protein, potassium, calcium, phosphate or urea.

4. Stimulated parotid saliva showed significant circadian rhythms in the concentrations of protein, sodium, potassium, calcium and chloride but not in phosphate or urea

5. Oral temperature showed a circadian rhythm which, like the salivary rhythms, was of a 24.0 hr periodicity.

INTRODUCTION

The literature on rhythms in salivary composition and flow rate has recently been reviewed in detail (Dawes, 1972). In most previous studies the results have not been subjected to the type of statistical analysis which gives quantitative information about the presence and characteristics of rhythms. In addition, most studies have not adequately standardized the normal physiological variables which can influence salivary composition. These variables include flow rate, duration of stimulation, nature of the stimulus, the method of saliva collection and the time interval between saliva collections. When saliva collections are made too

frequently, or just after meals, a serial dependency of sampling occurs and in studies by most workers frequent saliva collections were made from several subjects over a single 24 hr time span. A previous study (Dawes, 1972) showed that to avoid one collection of saliva influencing the composition of saliva collected subsequently, collections of stimulated saliva should not be made more frequently than every 1 or 2 hr. To determine the presence and characteristics of salivary rhythms it would seem preferable to make relatively few saliva collections per day for a much longer time span than 24 hr.

The best established rhythm in the composition of whole saliva is that for sodium concentration, maximal sodium values occurring early in the morning (Grad, 1952, 1954; de Traverse & Coquelet, 1952; Pawan, 1955; Prader, Gautier, Gautier, Näf, Semer & Rothschild, 1955; Kral, Grad & Hunzinger, 1959). Palmai & Blackwell (1965) and Palmai, Blackwell, Maxwell & Morgenstern (1967) have reported a circadian rhythm for salivary flow rate with the highest value being at 04.00 hr and the minimum at 20.00 hr. However, Peck (1959) and Chauncey, Feller & Shannon (1963) have recorded higher flow rates in the afternoon than in the morning.

There are two basically different methods of collecting stimulated saliva from a single gland such as the parotid. The first method has been used exclusively by previous investigators and involves the use of a constant stimulus. At each saliva collection the subject may, for instance, be given the same weight of wax to chew or sour lemon drop to suck on. The main advantage of this procedure is that information is obtained about differences in flow rate at different times of day. A major disadvantage is that the interpretation of changes in salivary composition is very difficult since the variations in composition could be due to (*a*) changes in plasma composition with time of day, (*b*) changes in the secretory mechanisms of the gland or (*c*) differences in mean flow rate or irregularities in flow rate during the saliva collections.

The second method involves the maintenance of a constant flow rate as described by Dawes (1967*a*). Although this technique gives no information about possible variations in stimulated flow rate with time of day it greatly facilitates the interpretation of changes in salivary composition since flow rate is no longer a variable. According to Wesson (1964) and Mills (1966), plasma electrolyte concentrations other than that of phosphate do not show significant circadian rhythms in humans. Thus, with the constant flow rate technique, any variations in salivary composition with time of day probably reflect variations in the secretory mechanisms of the glands.

For the present experiment eight subjects were studied for time spans of between 4 and 26 days.

METHODS

Collection of saliva

Unstimulated whole saliva was collected for a 5 min time span. The subject, seated at a low table, swallowed residual saliva present in the mouth before the beginning of the collection and then, with the head down and mouth slightly open, saliva was allowed to drip from the lower lip into a small plastic funnel and from there into a graduated 10 ml. centrifuge tube in ice water. In the last few seconds of the 5 min, saliva accumulated in the mouth was spat out into the plastic funnel. No other conscious movements of the oral musculature were made during the collection.

Stimulated left parotid saliva was collected with the aid of a Lashley cannula (Shannon & Chauncey, 1967). With 'sour lemon drops' as the gustatory stimulus a constant flow rate of 1·0 ml./min was maintained for 10 min as described previously (Dawes, 1967*a*). Briefly, saliva was collected into the graduated centrifuge tube positioned in front of a mirror. By observation the subject was able to calculate the flow rate with the aid of a stopwatch and regulate the degree of sucking on the sour lemon drops to maintain a constant flow rate. Since the composition of saliva undergoes marked changes in the first few minutes of stimulation (Dawes, 1969), only the 5·0 ml. collected during the 6-10 min of stimulation was retained for analysis.

The parotid saliva was collected beginning about 2 min after the collection of unstimulated whole saliva and the time of sampling was recorded as that at the end of the collection of unstimulated saliva. Saliva was collected five times daily at arbitrary but convenient times of about 07.00, 11.00, 14.00, 17.00 and 22.00. Most of the collections at 07.00, 17.00 and 22.00 hr were done in the subject's homes. Meals were consumed immediately after the collections at 07.00, 11.00, 17.00 and 22.00 hr in order to ensure freedom from salivary stimulation for a few hours before the test collections. Sleeping hours were from about 23.00 to 06.30 hr.

Oral temperature was recorded with a clinical thermometer about six times each day for at least a month, including the days during which the saliva collections were made. The thermometers were calibrated to read in ° F but in the results, temperature is given as °C. Halberg, Reinhardt, Bartter, Delea, Gordon, Reinberg, Ghata, Halhuber, Hofmann, Günther, Knapp, Pena & Garcia-Sainz (1969) have noted the value of recording body temperature as an internal standard in studies of human circadian rhythms.

Eight young adult (three female) subjects collected saliva, one for 4 consecutive days, three for 11 days, three for 4 and 11 days and one for 3, 4 and 26 days as illustrated in Fig. 1.

Analytical techniques

The unstimulated whole saliva was centrifuged at 49,000 *g* and the supernatant was retained for analysis. The concentrations of total protein and inorganic phosphate were determined in both types of saliva by colorimetric techniques, chloride by a coulometric method and sodium, potassium and calcium by atomic absorption spectroscopy as described previously (Dawes, 1967*b*, 1969). Urea was analysed by the method of Coulombe & Favreau (1963), in saliva collected from six of the eight subjects. Preliminary studies showed that when saliva was kept in ice water for 3 days there was no change in the measured concentrations of the above components except for urea. However, most samples of saliva were analysed on the day following saliva collection and immediately after each saliva collection an aliquot was added to tungstic acid in preparation for urea analysis.

Statistical analysis

A least square cosine wave was fitted to the data with the aid of a computer as outlined by Halberg, Engeli, Hamburger & Hillman (1965). A cosine wave is described by the equation:

$$f(t) = C_0 + C.\cos\left(\frac{2\pi t}{\tau} + \phi\right),$$

where t = time,

C_0 = mean value of series,

C = amplitude of rhythm,

τ = trial period under study,

ϕ = phase angle of acrophase.

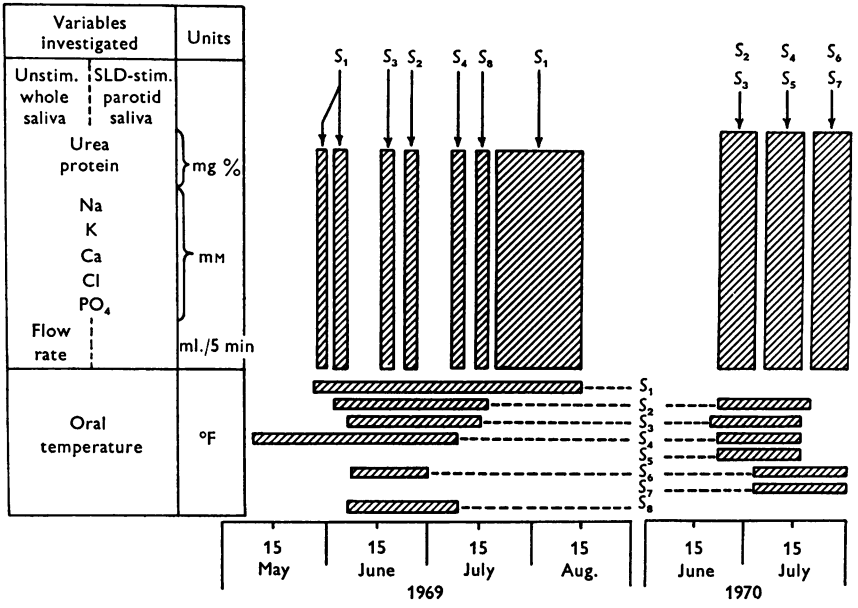

Fig. 1. Collection schedule of oral temperature, unstimulated whole saliva and sour lemon drop-stimulated (SLD) parotid saliva for eight subjects. S_n = subject number n.

In checking for circadian rhythms, values of τ at intervals of 0·1 hr between 20 and 28 hr are tested by the computer and for each value of τ the values of C_0, C and ϕ are simultaneously adjusted to give the best-fitting cosine wave. The period of best fit for the data is that for the cosine wave which accounts for the greatest portion of the total variance. The computer programme was designed to accept data recorded at irregular time intervals.

For each subject and each salivary component analysed, an estimate of the level, amplitude, phase angle and period of the best-fitting cosine wave was obtained. For those subjects who had collected saliva for two or more separate time spans the data were pooled.

For a given component, values of C and ϕ for the best-fitting cosine wave with a 24·0 hr period, for each subject, were subjected to cosinor analysis as described by Halberg, Tong & Johnson (1967). This computer programme derives mean, weighted amplitude and phase estimates together with 95% confidence limits (as an error ellipse) and plots the results, using polar coordinates, directly on to microfilm. The rhythm is only statistically significant for the group when the error ellipse does not overlap the pole.

RESULTS

The results for the best-fitting cosine wave with a 24.0 hr period for unstimulated whole saliva are summarized in Table 1 and those for oral temperature and stimulated parotid saliva in Table 2. The cosinor analyses of the most prominent rhythms are shown in Figs. 2–6.

In Tables 1 and 2, ϕ is presented in degrees, the 24 hr day being 360° and each hour 15°. Thus, for example, a value of $\phi = -231{\cdot}6°$ for flow rate in Table 1 indicates that peak unstimulated salivary flow rate occurred at 15.26 hr. In averaging values of ϕ, which is a circular function with possible values from 0 to 360°, it is important that when the mean acrophase is close to midnight and the individual values span midnight, the values may need an initial transformation before they are averaged. Thus $-3°$ rather than $-183°$ would be the correct average of $-359°$ and $-7°$. When the values of ϕ are widely scattered, the mean value is of little significance and this is reflected in a large standard error.

For rhythms which showed group significance by the cosinor method, the period of the best fitting cosine wave for the majority of the subjects was between 23·9 and 24·1 hr. The subject studied for only 4 days showed, as might be expected, the least significant rhythms.

When the best-fitting cosine wave was found to have a period of say 23·9 hr, the collection spans were too short to show that this differed significantly from 24·0 hr and thus all the results for C_0, C, ϕ and % C.V. in Tables 1 and 2 are for best-fitting cosine waves of period 24·0 hr, phase referenced to local midnight.

Cosinor analysis of the results for urea did reveal significant rhythms for both types of saliva. However, I believe this to be due to a fortuitous agreement of the ϕ values (which tends to produce a significant result by the cosinor analysis) since for only one of the subjects was the period of the best-fitting cosine wave between 23·9 and 24·1 hr and only one subject for parotid saliva and two for unstimulated saliva showed significant individual rhythms.

After the statistical analysis of the results had been completed further collections of parotid saliva were made from S_1, the only difference from the previous collections being that samples were also collected after the subject was weakened by an alarm clock in the middle of the night.

TABLE 1. Circadian rhythm parameters for flow rate and composition of unstimulated whole saliva

	Level (C_0) ± S.E.	Amplitude (C) ± S.E.	Acrophase (ϕ) ± S.E. (in degrees)	No. of subjects with $23{\cdot}9 \geqslant \tau \leqslant 24{\cdot}1$ hr	% C.V.† ± S.E.	No. of subjects with a sig. rhythm ($P < 0{\cdot}05$)	Group significance by cosinor
Flow rate (ml./5 min)	$2{\cdot}43 \pm 0{\cdot}46$	$0{\cdot}94 \pm 0{\cdot}17$	$-231{\cdot}6 \pm 11{\cdot}2$	6	$34{\cdot}2 \pm 4{\cdot}8$	8	**
Sodium (mM)	$6{\cdot}2 \pm 0{\cdot}9$	$4{\cdot}3 \pm 1{\cdot}2$	$-70{\cdot}0 \pm 2{\cdot}6$	7	$45{\cdot}7 \pm 6{\cdot}6$	7	*
Sodium/potassium	$0{\cdot}29 \pm 0{\cdot}05$	$0{\cdot}18 \pm 0{\cdot}06$	$-66{\cdot}9 \pm 3{\cdot}3$	7	$46{\cdot}0 \pm 7{\cdot}3$	7	*
Chloride (mM)	$17{\cdot}4 \pm 1{\cdot}4$	$4{\cdot}8 \pm 0{\cdot}9$	$-74{\cdot}0 \pm 6{\cdot}4$	7	$41{\cdot}9 \pm 3{\cdot}8$	8	**
Protein (mg %)	$223{\cdot}0 \pm 20{\cdot}0$	$63{\cdot}5 \pm 15{\cdot}6$	$-119{\cdot}5 \pm 36{\cdot}5$	6	$21{\cdot}6 \pm 4{\cdot}2$	7	N.S.
Potassium (mM)	$21{\cdot}6 \pm 1{\cdot}2$	$1{\cdot}1 \pm 0{\cdot}2$	$-210{\cdot}2 \pm 44{\cdot}4$	3	$6{\cdot}6 \pm 2{\cdot}0$	2	N.S.
Calcium (mM)	$1{\cdot}75 \pm 0{\cdot}10$	$0{\cdot}18 \pm 0{\cdot}04$	$-137{\cdot}3 \pm 33{\cdot}6$	4	$18{\cdot}9 \pm 4{\cdot}1$	7	N.S.
Inorg. phosphate (mM)	$6{\cdot}14 \pm 0{\cdot}61$	$0{\cdot}66 \pm 0{\cdot}18$	$-325{\cdot}6 \pm 28{\cdot}2$	4	$10{\cdot}2 \pm 2{\cdot}9$	4	N.S.
Urea (mM)	$32{\cdot}2 \pm 2{\cdot}5$	$3{\cdot}7 \pm 0{\cdot}9$	$-357{\cdot}0 \pm 21{\cdot}6$	0	$11{\cdot}0 \pm 4{\cdot}3$	2	—

* = $P < 0{\cdot}05$. ** = $P < 0{\cdot}01$.
† = % circadian variance (% of total variance accounted for by circadian rhythm).
N.S. = not significant.

TABLE 2. Circadian rhythm parameters for oral temperature and composition of sour lemon drops-stimulated parotid saliva (1 ml./min)

	Level (C_0) ± S.E.	Amplitude (C) ± S.E.	Acrophase (ϕ) ± S.E. (in degrees)	No. of subjects with $23{\cdot}9 \geqslant \tau \leqslant 24{\cdot}1$ hr	% C.V.† ± S.E.	No. of subjects with a sig. rhythm ($P < 0{\cdot}05$)	Group significance by cosinor
Oral temp. (° C)	36·72 ± 0·07	0·38 ± 0·07	−250·3 ± 4·0	8	34·2 ± 4·1	8	***
Protein (mg %)	270·5 ± 29·2	81·2 ± 14·2	−238·4 ± 6·7	5	31·9 ± 6·9	6	**
Sodium (mM)	29·0 ± 4·2	4·2 ± 0·7	−75·7 ± 9·2	6	23·2 ± 5·7	6	**
Potassium (mM)	22·0 ± 0·8	1·4 ± 0·2	−260·4 ± 9·4	5	22·4 ± 4·2	7	**
Sodium/potassium	1·38 ± 0·23	0·28 ± 0·04	−76·4 ± 8·2	7	30·8 ± 5·5	8	**
Calcium (mM)	1·02 ± 0·07	0·04 ± 0·01	−288·0 ± 13·8	3	7·2 ± 2·0	3	*
Chloride (mM)	17·1 ± 2·8	2·0 ± 0·5	−76·5 ± 8·1	5	15·8 ± 4·3	5	*
Inorg. phosphate (mM)	3·16 ± 0·27	0·11 ± 0·03	−169·3 ± 34·8	1	3·5 ± 1·3	1	N.S.
Urea (mg %)	26·7 ± 1·7	2·0 ± 0·2	−335·0 ± 9·4	1	8·4 ± 1·0	1	—

* = $P < 0{\cdot}05$. ** = $P < 0{\cdot}01$. *** = $P < 0{\cdot}001$.
† = % circadian variance (% of total variance accounted for by circadian rhythm).
N.S. = not significant.

Samples collected at this time contained the lowest protein and potassium and highest sodium concentrations for those days, in agreement with the results calculated from the 26-day collections in which no saliva collections were actually made during the middle of the night. Representative results are shown in Table 3.

From Tables 1 and 2 it is apparent that even the most significant circadian rhythms accounted for less than 50% of the total variance, suggesting that other variables which can influence salivary composition still remain to be identified.

DISCUSSION

Oral temperature displayed the most pronounced circadian rhythm, as determined by cosinor analysis (Fig. 2), with the acrophase at 16.41 hr, a value falling in the normal range obtained by other workers (Halberg *et al.* 1969).

The flow of unstimulated whole saliva showed a very marked circadian rhythm of high amplitude (Fig. 3) with the acrophase at 15.26 hr. The position of the acrophase differs from that at 04.00 hr found by Palmai & Blackwell (1965) in normal subjects tested for 4 hourly over a single 24 hr time span. However, they estimated saliva volume by the increase in weight of dental cotton-wool rolls placed in the mouth for 2 min spans, a collection procedure which would undoubtedly stimulate salivary flow rate. Peck (1959) using a technique similar to that of Palmai & Blackwell found peak flow rates about 17.00 hr.

It is possible that antidiuretic hormone could contribute to the rhythm in flow rate as maximum ADH output occurs during the night (Mills, 1966). Junqueira, Fava-de-Moraes & Toledo (1967) reported that ADH injection reduced salivary secretion in several species, including the human, and Holmes (1964) reported a slight reduction in salivary flow rate on injection of ADH in humans.

It is well known that during sleep the salivary flow rate is extremely low (Schneyer, Pigman, Hanahan & Gilmore, 1956; Lear, Flanagan & Moorrees, 1965), in fact much lower than when samples are collected from subjects wakened in the middle of the night. The circadian rhythm in flow rate is of particular clinical significance and is illustrated in a more conventional manner in Fig. 7. Because salivary flow rate drops so markedly during sleep, it would seem that the most important time to carry out oral hygiene procedures would be before going to bed. During the day, acid formed in the dental plaque is neutralized by virtue of saliva acting as a diluent, by the buffers in saliva and possibly by the activity of the pH-rise factor in saliva (Kleinberg, 1970). During the night these protective effects of saliva would largely be absent.

The protein concentration in parotid saliva showed a circadian rhythm of high amplitude (Fig. 4), but there is no obvious explanation for the position of the acrophase. The results fit in with those of Ferguson, Krahn & Hildes (1958) but do not support the findings of Ferguson, Elliott &

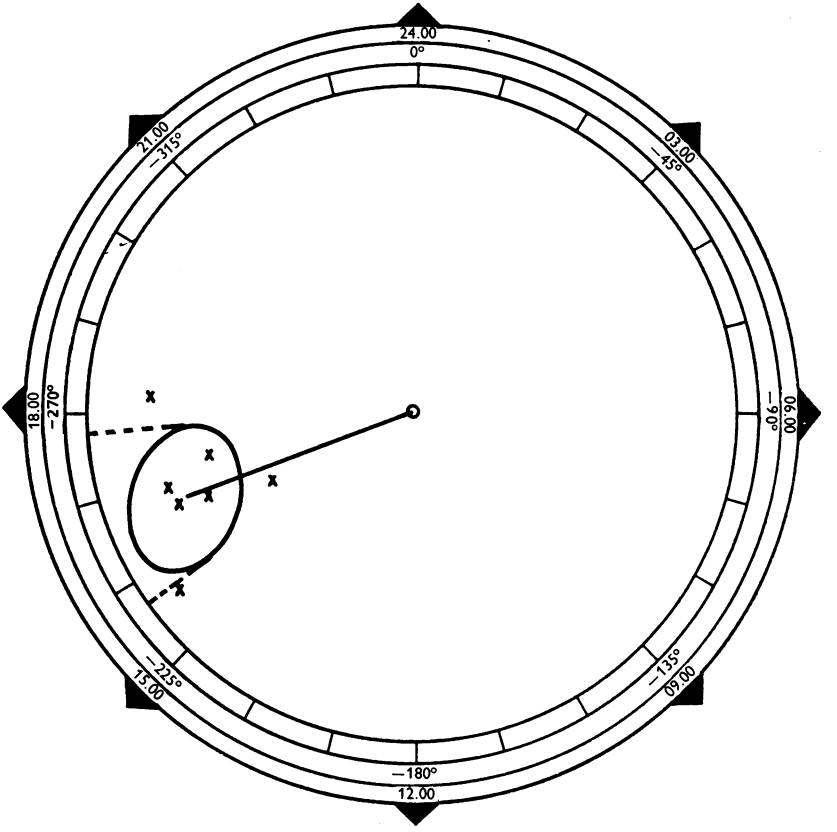

Fig. 2. Cosinor summary of circadian rhythm in oral temperature. The length of the line from the origin represents the amplitude of the rhythm which was 0·37° C. The error ellipse indicates 95% confidence limits for amplitude and phase and the dashed lines continue the tangents from the origin to the ellipse. The crosses indicate individual subject values for amplitude and phase. The outer circle indicates clock hours and the inner two the phase angle in degrees where 360° = 24 hr.

Potts (1969) who noted peak protein concentrations at midnight when collections of parotid saliva were made every 2 hr for a 24 hr time span with the 'constant stimulus' technique. Shannon & Segreto (1968*a*) found peak protein concentrations at 10.00 hr in a study on parotid saliva col-

lected by the constant stimulus method at 2-hourly intervals between 08.00 and 16.00 hr on a Monday to Friday basis for 4 weeks.

The degree of correlation between protein and calcium concentrations in saliva has been discussed previously (Windeler & Shannon, 1966; Dawes, 1967*b*). It is noteworthy that although the total protein concentration in parotid saliva showed a circadian rhythm of high amplitude, the rhythm in calcium concentration was of extremely low amplitude suggesting that there is little correlation between protein and calcium concentrations. Only three of the eight subjects showed a significant individual rhythm in parotid calcium concentration.

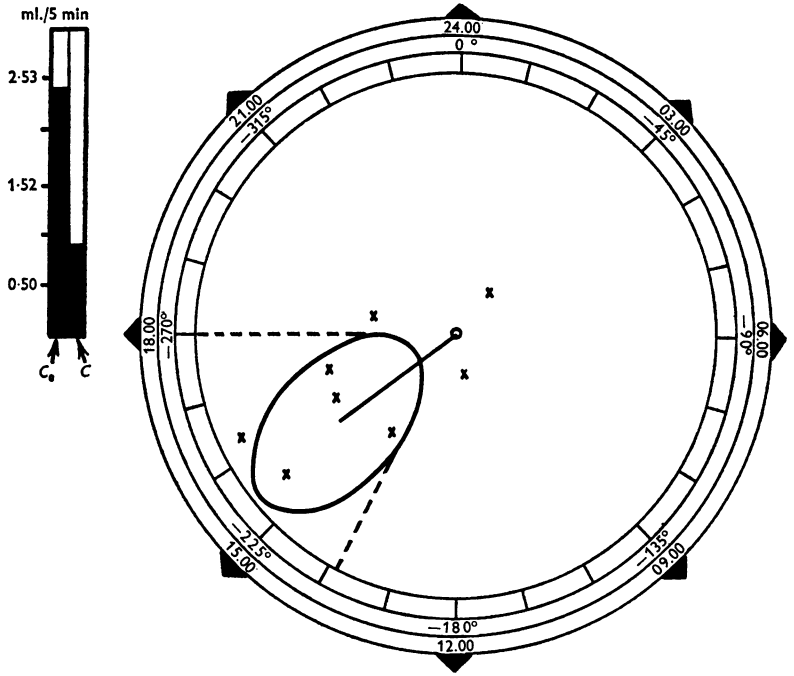

Fig. 3. Cosinor summary of circadian rhythm in unstimulated salivary flow rate.

Although seven of the eight subjects showed significant individual rhythms in both total protein and calcium concentrations in unstimulated whole saliva, the acrophases were widely scattered and cosinor analysis revealed non-significant group rhythms. Protein in whole saliva may be derived not only from the salivary secretions but also from partially solubilized oral micro-organisms, desquamated epithelial cells and oral leucocytes. As the unstimulated saliva was centrifuged before analysis to

remove cellular débris it is possible that varying amounts of protein may have been precipitated in the presence of such débris and carried down during centrifugation.

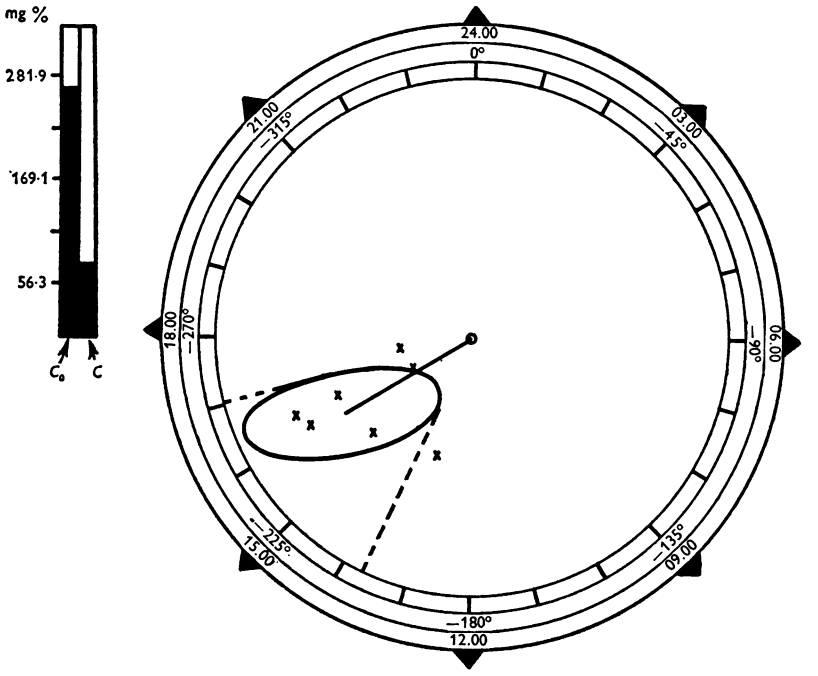

Fig. 4. Cosinor summary of circadian rhythm of protein concentration in stimulated parotid saliva.

For both types of saliva, peak sodium concentrations occurred around 05.00 hr contrary to the results of Ferguson *et al.* (1969) but in agreement with the results of other investigators (Grad, 1952; Kral *et al.* 1959; Shannon & Segreto, 1968*b*; Bissada & Haus, 1968) who noted peak sodium concentrations in early morning samples. The rhythm in the sodium concentration in unstimulated saliva is of particular interest as it is exactly 12 hr out of phase with the rhythm in flow rate and normally the sodium concentration is directly related to flow rate (Dawes, 1969). Henkin, Gill & Bartter (1963) have reported an increased taste sensitivity for sodium chloride in normal subjects tested in the afternoons as compared with the mornings, and the finding of minimum sodium and chloride concentrations in the afternoon may partially account for this.

Despite the large difference in level, the amplitudes of the sodium rhythms in the two types of saliva were almost identical.

The potassium results for the two types of saliva do not correspond in that the acrophase for stimulated parotid saliva was at 17.42 hr almost exactly 12 hr out of phase with the sodium rhythm (Fig. 5), whilst unstimulated whole saliva did not show a significant rhythm for potassium. Shannon & Segreto (1968*b*) noted peak potassium concentrations in the parotid samples collected at 14.00 hr.

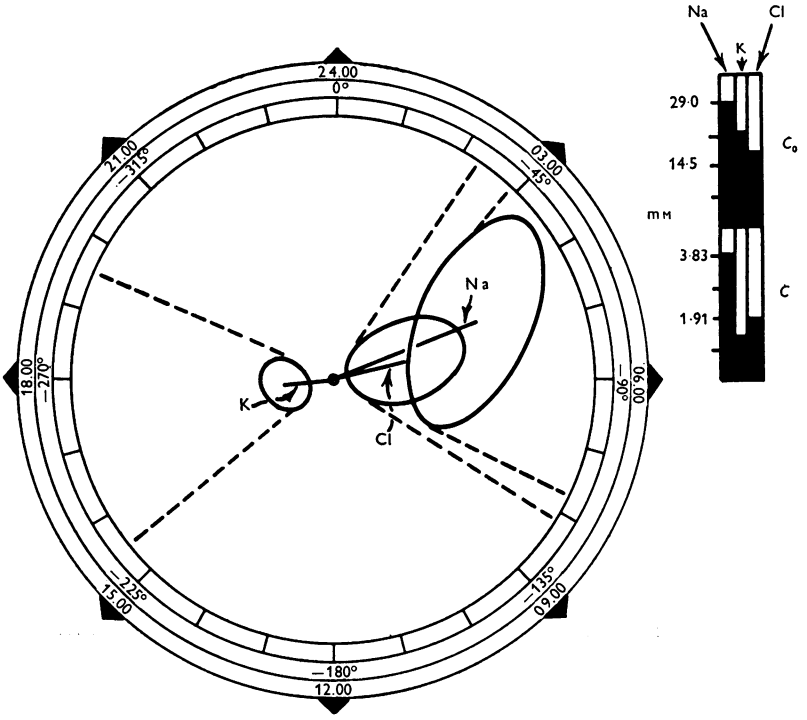

Fig. 5. Multiple cosinor summary of circadian rhythms in sodium, potassium and chloride concentrations in stimulated parotid saliva.

The sodium, potassium and chloride rhythms could be due to the influence of a rhythm in the plasma concentration of aldosterone as rhythms in salivary sodium concentration are not found in patients with Addison's disease (Pawan, 1955; Kral *et al.* 1959). Wesson (1964) and Wolfe, Gordon, Island & Liddle (1966) have stated that plasma aldosterone concentrations reach peak values in the forenoon and Blair-West, Coghlan, Denton & Wright (1967) have shown that aldosterone is the main hormone controlling sodium excretion in the sheep parotid gland. Steroids other than aldosterone are less likely to be involved as Mills (1966) and Katz & Shannon (1969) have reported that peak values in plasma cortisol and cortisone concentrations occur in the early morning. Mangos & McSherry

(1969), in a micropuncture study of parotid saliva from normal and adrenalectomized rats, some of which received supplements of D-aldosterone, showed that in the absence of aldosterone, salivary sodium levels were considerably elevated whereas potassium levels were only slightly lowered. The large amplitude for the sodium rhythm and the small amplitude for the potassium rhythm in parotid saliva (Fig. 5) are in conformity with the findings of Mangos & McSherry and the timing of the salivary rhythm is consistent with that for plasma aldosterone if changes in aldosterone concentrations take a few hours to be effective at the level of the salivary gland, as found by Blair-West *et al.* (1967) in the sheep.

The lack of a potassium rhythm in unstimulated whole saliva does not at first fit in with the above theory but, as whole saliva is derived from a number of separate glands, the secretions of which differ in composition (Dawes, 1972), it may be that the proportional contributions from the different glands vary sufficiently with time of day to distort the expected rhythm. The potassium concentration in unstimulated submandibular saliva tends to be considerably lower than that in unstimulated parotid saliva. Alternatively, since at low flow rates potassium concentration is inversely related to flow rate (Shannon, Suddick & Chauncey, 1969), the peak flow rate in the middle of the afternoon may reduce the expected potassium peak.

Some workers have studied sodium/potassium ratios in saliva and it may be seen from Tables 1 and 2 that there was a significant rhythm in sodium/potassium for both types of saliva. However, the fraction of total variance accounted for by the circadian rhythm was not significantly greater for the sodium/potassium rhythm than for the sodium rhythm alone.

It is unlikely that rhythms in plasma electrolyte concentrations can account for the observed salivary rhythms as Wesson (1964) and Mills (1966) have stated that, other than phosphate, plasma electrolytes do not display significant circadian rhythms. Despite a possible plasma phosphate rhythm neither type of saliva showed a rhythm in phosphate concentration. The absence of a phosphate rhythm in unstimulated saliva is of interest in view of the significant rhythm in flow rate and the normal inverse relation between flow rate and phosphate content (Shannon *et al.* 1969).

The chloride concentration in parotid saliva showed a rhythm of lower amplitude than that for sodium (Fig. 5), the minimum concentration occurring in the afternoon in agreement with the findings of Shannon & Segreto (1968*b*), but contrary to those of Ferguson *et al.* (1969). Fewer subjects showed a significant chloride rhythm in parotid than in unstimulated saliva. The subject studied for the longest time span (S_1) did not show a significant chloride rhythm in parotid saliva but showed a very

significant one in unstimulated saliva. However, the mean parotid chloride level in S_1 was unusually low (8·8 mM).

Bicarbonate was the only major electrolyte in saliva not studied directly but it may be deduced that any rhythms, if present, must have been of very low amplitude. In parotid saliva, the electrolytes showing rhythms with a reasonably large amplitude were sodium, potassium and chloride. It may be seen from Fig. 5 and also Table 2 that if the amplitudes of the rhythms for the three ions are summed, to test for an excess or deficit of anions, at no time of day is there a significant difference from zero. Similarly, for unstimulated whole saliva for which the only major electrolytes showing significant rhythms are sodium and chloride, these are in phase with each other and are of equal amplitude (Fig. 6 and Table 1).

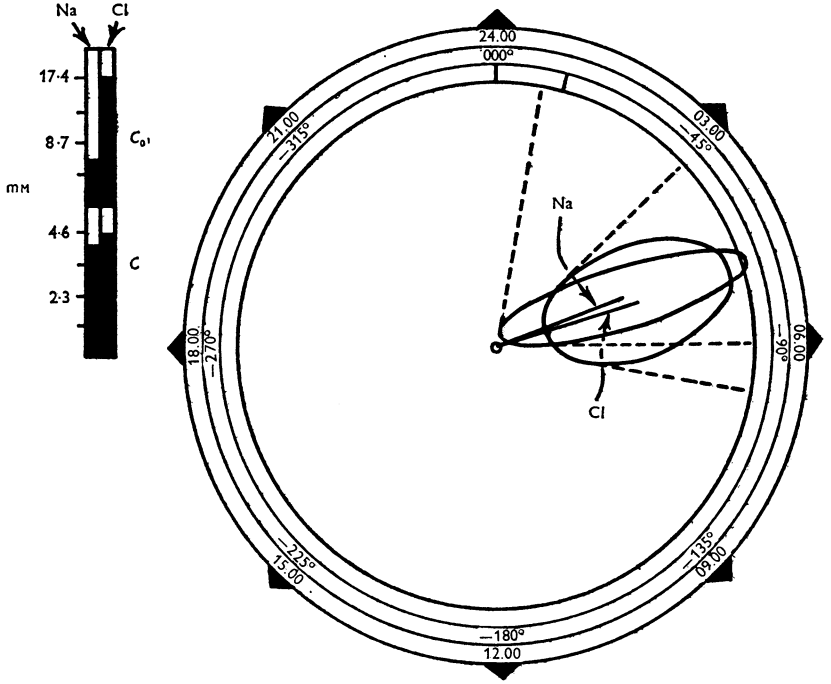

Fig. 6. Multiple eosinor summary of circadian rhythm in sodium and chloride concentrations in unstimulated whole saliva.

The presence of circadian rhythms in salivary flow rate and composition must influence the concept of normal values and in any study on saliva the time of day of sampling could have an important bearing on the results. Many investigators collect samples at the beginning of the working day, a time at which, for example, unstimulated flow rate and sodium concentrations are showing their most rapid rate of change. The variations in

salivary composition with time of day could mean that at certain times of day patients are particularly susceptible to oral disease. This concept has been discussed elsewhere (Dawes, 1972).

TABLE 3. Protein, sodium and potassium concentrations in sour lemon drops-stimulated left parotid saliva (1 ml./min)

Date	Time	Protein (mg %)	Na (mM)	K (mM)
16 October 1969	02.15	115	23·2	21·1
	07.10	185	21·3	23·0
	11.10	295	15·1	23·7
	13.55	300	15·4	24·2
	17.00	260	14·4	25·1
	23.00	155	18·4	23·3
17 October 1969	02.45	100	22·3	23·7
	07.35	165	19·9	22·3

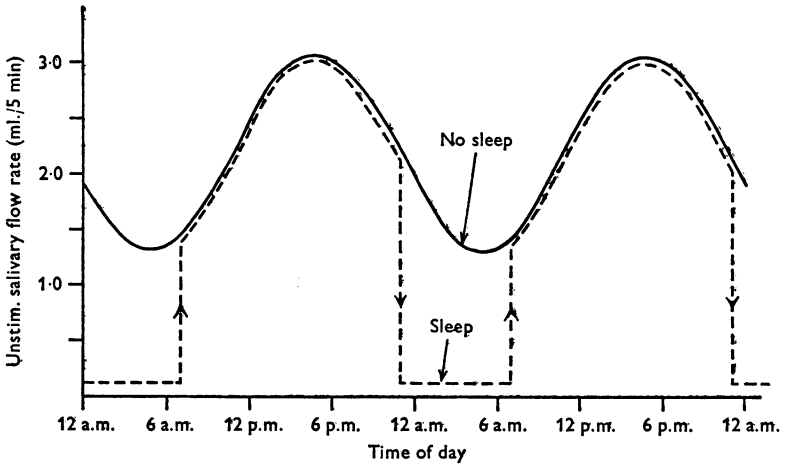

Fig. 7. The circadian rhythm in unstimulated salivary flow rate (continuous line) and the idealized effect of sleep (dashed line) from 23.00 to 07.00 hr.

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